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Dispersive liquid–liquid microextraction versus single-drop microextraction for the determination of several endocrine-disrupting phenols from seawaters

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ABSTRACT

Two liquid-phase microextraction procedures: single-drop microextraction (SDME) and dispersive liquid–liquid microextraction (DLLME), have been developed for the determination of several endocrine-disrupting phenols (EDPs) in seawaters, in combination with high-performance liquid chromatography (HPLC) with UV detection. The EDPs studied were bisphenol-A, 4-cumylphenol, 4-tertbutylphenol, 4-octylphenol and 4-*n*-nonylphenol. The optimized SDME method used 2.5 μL of decanol suspended at the tip of a micro-syringe immersed in 5 mL of seawater sample, and 60 min for the extraction time. The performance of the SDME is characterized for average relative recoveries of 102 ± 11%, precision values (RSD) < 9.4% (spiked level of 50 ng mL^{-1}), and detection limits between 4 and 9 ng mL^{-1}. The optimized DLLME method used 150 μL of a mixture acetonitrile:decanol (ratio 15.7, v/v), which is quickly added to 5 mL of seawater sample, then subjected to vortex during 4 min and centrifuged at 2000 rpm for another 5 min. The performance of the DLLME is characterized for average relative recoveries of 98.7 ± 3.7%, precision values (RSD) < 7.2% (spiked level of 20 ng mL^{-1}), and detection limits between 0.2 and 1.6 ng mL^{-1}. The efficiencies of both methods have also been compared with spiked real seawater samples. The DLLME method has shown to be a more efficient approach for the determination of EDPs in seawater matrices, presenting enrichment factors ranging from 123 to 275, average relative recoveries of 110 ± 11%, and precision values (RSD) < 14%, when using a real seawaters (spiked level of 3.5 ng mL^{-1}).

1. Introduction

Several environmental contaminants, termed as endocrine disrupting chemicals (EDCs), can interfere with the function of the endocrine system in living organisms [1,2]. Chemical exposure to EDCs has been linked to neurological and reproductive effects on fish and wildlife [1,3], and may also affect human fertility [4]. These findings have raised public concern over their environmental and human health effects. Sewage treatment plants (STP) effluent outfalls constitute significant sources of EDCs to the receiving surface, coastal waters and regional environments [5,6], thus increasing the risk of exposure. Among the increasing list of substances classified as EDCs, an important attention has been paid to a selected group of endocrine-disrupting phenols (EDPs), alkylphenols (APs), including nonylphenol (NP) and bisphenol-A (BPA), due to their wide presence in household and industrial processes [5].

A number of analytical methods have been employed to determine EDPs in the aquatic environment, including drinking water, river water and wastewater [7–10]. Limited data are present in the literature about EDC determinations in marine water [11–13], due to the complexity of the salty matrix. Many conventional methods used to obtain quantitative extractions for APs are characterized as being labor-intensive and time-consuming, because in most cases derivatization and/or solvent-exchange steps are needed [6,13,14]. The conventional extraction methods are also hazardous to human health as they often use large amounts of organic solvents, which are known for being hazardous, flammable, and damaging to the environment. These disadvantages have been the basis of a trend to develop analytical methods aimed at eliminating, or at least minimizing, the organic solvent consumption in sample preparation [15]. Solid-phase microextraction (SPME) [16] and liquid-phase microextraction (LPME) [17,18] can be highlighted among them.

Single-drop microextraction (SDME) [18–20] is a liquid-phase microextraction technique which consists of the suspension of a drop of a solvent (typically few micro-liters of an organic solvent) at the tip of a micro-syringe. The drop, in which analytes suffer partitioning, is exposed to the sample, then retracted into the syringe and transferred to an appropriate analytical system. SDME can be conducted in headspace or in direct immersion mode, and it includes many advantages such as low cost and little sample and solvent consumption. It combines extraction, preconcentration and sample introduction in one step. In addition to this, the possibility of carry over between analyses is negligible.

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Dispersive liquid–liquid microextraction (DLLME) is another recent microextraction technique [21,22] which employs a mixture of a non-water-miscible extraction solvent and a water miscible polar disperser solvent. Analytes experience enrichment in the low volume of extraction solvent which is dispersed into the bulk aqueous solution, and separated by centrifugation. DLLME is a successful extraction technique due to the high contact surface of fine droplets of extraction solvent and analytes, which speeds up the mass transferring processes of analytes from aquatic phase to the extractant organic phase. DLLME presents characteristics of homogeneous liquid–liquid extraction and cloud-point extraction. Some advantages of DLLME are simplicity of operation, rapidity, low sample volume, low cost and high enrichment factor.

Main analytical applications of these two liquid-phase microextraction techniques, SDME and DLLME, have been conducted in combination with GC, HPLC and AAS [20–28].

The main purpose of this work was to carry out a comparison of the two liquid-phase microextraction techniques: single-drop microextraction and dispersive liquid–liquid microextraction, for the determination of a group of endocrine-disrupting phenols in seawaters close to a STP plant. To our knowledge, this is the first report in which both microextraction procedures are used in the analysis of this group of EDPs from seawaters.

2. Experimental

2.1. Reagents

The endocrine-disrupting phenols (EDPs) used in this study were bisphenol-A (BPA), 4-cumylphenol (4-CP), 4-tertbutylphenol (*t*-BP), 4-octylphenol (OP), and 4-*n*-nonylphenol (NP). They were all supplied by Sigma–Aldrich Chemie GmBH (Steinheim, Germany) except for NP, which was supplied by Alfa-Aesar (Karlsruhe, Germany). Individual standard solutions of these EDPs were prepared in acetonitrile of HPLC gradient quality (Merck, Darmstadt, Germany), with individual concentrations of 400 mg L^{-1}. Working standard solutions were prepared by adequate dilutions of these individual standards. 1-Decanol of 99% purity was obtained from Sigma–Aldrich.

Preparation of the artificial seawater was carried out by dissolving the following salts in 1 L of deionized water: NaF (3 mg), KBr (100 mg), $Na_2SiO_3{\cdot}9H_2O$ (20 mg), $SrCl_2{\cdot}H_2O$ (20 mg), KCl (3 mg), $MgCl_2{\cdot}6H_2O$ (10.780 g), $NaHCO_3$ (200 mg), H_3BO_3 (30 mg), $CaCl_2{\cdot}2H_2O$ (1.470 g) and NaCl (23.500 g) [29]. All of these pro-analysis salts were supplied by Merck. Deionized water was obtained from a Milli-Q gradient A10 system (Millipore, Watford, UK).

Real seawater samples came from two quite different sampling areas in Tenerife Island: the coasts of Candelaria and the surroundings of a petroleum refinery, where effluent waters are discharged from a STP. Amber glass bottles 100 mL in volume were used for sampling. Seawaters from Candelaria characteristically presented a content of 3.8 mg L^{-1} in suspended solids, a pH of 8.1, a content of dissolved oxygen of 8.0 mg L^{-1}, and a salinity value of 36.2 g L^{-1}. Seawaters close to the refinery had a variable content in terms of suspended solids, from 3.4 to 5.6 mg L^{-1}, salinity values, from 36.3 to 36.7 g L^{-1}, and dissolved oxygen, from 7.1 to 8.3 mg L^{-1} (depending on the sample location), and an average pH of 8.2.

Acetonitrile of gradient quality (Merck) and deionized water were used for HPLC analysis.

2.2. Instrumentation

The HPLC equipment consisted of a gradient system L-2130 Merck Hitachi Pump (supplied by Merck) and a Rheodyne valve (Supelco, Bellefonte, PA, USA) with a 20 μL loop. The detection of EDPs was carried out using a Waters Lambda Max 481 UV detector (Milford, MA, USA). The analytical column was a C18 Res Elut HPLC Column (5 μm, 150 mm × 4.6 mm) supplied by Varian (Harbor City, CA, USA), and protected by a Pelliguard LC-18 guard column (Supelco). Data were acquired with the Autoanalysis 2.4 software (Sciware, victor.cerda@uib.es). The HPLC method used for the separation and determination of the EDPs consists of a gradient elution procedure with a UV detector operating at 228 nm. For the mobile phase, a mixture of acetonitrile and water was used at a flow-rate of 1 mL min^{-1}. A linear gradient was employed from 50 to 80% of acetonitrile over 8 min, and then 80% of acetonitrile was maintained for 9 min.

A vortex model reax-control from Heidolph Instruments GmbH (Schwabach, Germany) and a centrifuge model EBA3 S from Hettich-Zentrifugen (Tuttlingen, Germany) were used in all DLLME experiments. A magnetic stirrer model Ikamag RCT basic (IKA Werke GmbH, Staufen, Germany) was used in all SDME experiments.

The Statgraphic (Statistical Graphics, Rockville) software package Version 5.1 was used for the statistical treatment.

2.3. Procedures

The syringe used for single-drop microextraction experiments, dispersive liquid–liquid microextraction experiments, and sample injection, was 25 μL in volume and from Hamilton (Bonaduz, Switzerland).

In SDME experiments, the vial was placed on the magnetic stirrer. The optimized technique is performed by suspending a 2.5 μL drop of decanol at the tip of the 25 μL micro-syringe immersed in the 5 mL of aqueous solution (7 mL vial) stirred at 100 rpm. The aqueous solution was a real seawater sample (spiked or not) or an artificial seawater sample (spiked). Following sample extraction, the magnetic stirrer was switched off and the drop was withdrawn into the syringe. Then, acetonitrile (Scharlau) was withdrawn into the syringe to complete around 20 μL. After the needle tip was carefully cleaned with a tissue, the content of the syringe was injected into the HPLC for analysis. The overall SDME method calibration was performed using artificial seawater calibration solutions submitted to the same SDME procedure described above. The agitation for the SDME extractions was performed by using PTFE stir bars. Sorption of EDPs can take place on PTFE stir bars, and so they were carefully rinsed after use with acetone, then methanol, then acetonitrile and finally with deionized water to avoid memory effects. The stir bars were also daily sonicated for 10 min with acetonitrile.

In DLLME experiments, 5 mL of real seawater samples (spiked or not) or artificial seawater samples (spiked) are placed in a glass tube of 8 mL. Then, 150 μL of an adequate mixture acetonitrile:decanol is rapidly injected to the sample. The optimum ratio acetonitrile:decanol was 15.7 (v/v). Under the optimized procedure, the mixture is vigorously shaken during 4 min using the vortex at 2000 rpm. Following extraction, the tube is subjected to centrifugation during 5 min at 3600 rpm. Afterwards, the upper phase of 1-decanol containing the extracted EDPs (~19 μL) is removed using the Hamilton syringe. The overall DLLME method calibration was performed using artificial seawater calibration solutions submitted to the same DLLME procedure described above.

3. Results and discussion

3.1. Optimization of the SDME method

Several considerations must be taken into account to select an adequate extractant solvent for a SDME application in direct